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Risk Assessment for Neurobehavioral Toxicity

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A study by the National Academy of Sciences/National Research Council (NAS/NRC) found neurobehavioral toxicity to be one of the areas where almost no data are available for the assessment of toxicity. Using the NAS/NRC report and a data base from the American Conference of Government Industrial Hygienists (ACGIH), an estimate of the number of neurobehavioral toxins in commercial chemicals can be made. Although the assumption made in making such a calculation may be invalid, the exercise suggests that the number of neurobehavioral toxins may be quite large. There does seem to be general agreement as to what type of neurobehavioral test procedures are appropriate for regulatory purposes. Select committees have consistently recommended the use of test batteries that include schedule-controlled behavior, motor activity, and neuropathological examination following *in vivo* perfusion, for regulatory purposes. Alkyltin data developed from such a battery were applied to the risk assessment model employed by the United States Environmental Protection Agency (EPA) in their calculations of acceptable daily intake. Using this test battery and the EPA risk assessment model, the acceptable daily intake calculated is of the same order of magnitude as the total limit values established by the ACGIH. A number of special issues in neurobehavioral toxicity also are discussed, including the definition of adverse neurobehavioral toxic effects, species extrapolation, correlation of behavior and neuropathology, alternative methods, and quality of life issues.

Introduction

In 1980, the National Toxicology Program commissioned the National Academy of Sciences/National Research Council (NAS/NRC) to estimate the number of chemicals in the environment that produce human health risks and to develop a priority system for the toxicity testing of chemicals that might present risks to humans. In attempting to fulfill these obligations, the NAS/NRC appointed select committees to prepare a report (*1*). From various lists of chemicals, one of these committees defined a select universe of approximately 65,000 chemicals as a base for the study. A stratified, random sample of 100 chemicals was carefully selected to allow generalization from the sample to the select universe. The sample included pesticides, drugs, cosmetics, and chemicals in commerce.

In the second stage of the NAS/NRC report, the Committee on Toxicity Data elements developed guidelines for determining the quality of individual toxicity studies reported in the literature and then reviewed the data base for each of the 100 chemicals in the sample according to the guidelines. Among several areas where data adequate for the evaluations of toxicity was frequently unavailable was neurobehavioral toxicity. Table 1 shows the proportion of chemicals by chemical class for which data adequate for the evaluation of neurobehavioral toxicity were available. Clearly, there are available few data in the literature for evaluation of neurobehavioral toxicity for any of the classes of chemicals.

Table 1. Chemicals by chemical category with neurobehavioral toxicity data adequate for evaluation of neurobehavioral toxicity.[a]

Chemical category	% With prescribed minimal neurobehavioral toxicity data
Pesticides and pesticide ingredients	1–9
Cosmetic ingredients	0
Drugs and formulation ingredients	20–29
Food additives	10–19
Chemicals in commerce, >1 million lb/yr	0
Chemicals in commerce, <1 million lb/yr	10–19
Chemicals in commerce, amount unknown	0

[a] Data are from NAS/NRC (*1*).

The NAS/NRC report did not attempt to determine what proportion of chemicals in the select universe might produce neurobehavioral toxicity, as the report focused only on the question of whether or not data meeting the test guidelines for evaluating toxicity were available and not on whether neurobehavioral toxicity tests predicted neurobehavioral toxicity for a given chemical. Thus, no estimate can be made of the number

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of potential neurobehavioral toxins in the select universe on the basis of the NAS/NRC report.

Anger (*2*) has provided a data base, which when combined with the NAS/NRC report may provide a rough estimate of the potential number of neurobehavioral toxins in the select universe. Anger reviewed the American Conference of Government Industrial Hygienists (ACGIH) threshold limit values (TLVs). Although the ACGIH TLVs are voluntary, several agencies have adopted them for regulatory purposes. Anger reviewed the ACGIH documentation book to determine the basis for the TLVs. He found that 167 of the 588 (28.4%) chemicals for which TLVs have been set were determined all or in part on the basis of direct nervous system effects.

If the assumption is made that Anger's analysis of the ACGIH TLVs applies to all chemical classes, the NAS/NRC data can be used as an estimate of the number of chemicals in each chemical class and that value, when multipled by 28.4%, gives a gross estimate of the number of neurotoxic chemicals in each class. In making this calculation, the total number of chemicals in each category of the select universe has been used rather than the number of chemicals without adequate neurobehavioral toxicity testing, since the NAS/NRC report makes no estimate of the frequency of neurobehavioral toxicity among those chemicals that met the guidelines for adequate neurobehavioral toxicity testing. The data based on the NAS/NRC sample and Angers data are shown in Table 2. According to this estimate, the predicted number of chemicals that produce neurobehavioral toxicity is in excess of 18,000.

Obviously, some of the assumptions made in making these calculations are not likely to be valid. For example, the drugs that did not meet the NAS/NRC test guidelines but have been approved by the Food and Drug Administration and have been widely used by the human population are unlikely to match the 28.4% level of neurobehavioral effect that Anger found among industrial chemicals. The purpose of making the calculation was not to establish the accuracy of the estimate of 18,000 neurobehavioral toxins, but rather to suggest that there may be a large number of neurobehavioral toxins to which the human population is potentially exposed.

Table 2. Estimated number of neurobehavioral toxins.[a]

Chemical category	Number in category	Estimated number of neurobehavioral toxins
Pesticides and pesticide ingredients	3350	951
Cosmetic ingredients	3410	968
Drugs and formulation ingredients	1815	515
Food additives	8627	2450
Chemicals in commerce, > 1 million lb/year	12680	3652
Chemicals in commerce, < 1 million lb/year	13911	3951
Chemicals in commerce, amount unknown	21752	6178
Total	65,725	18,665

[a] Based on NAS/NRC (*1*) and Anger (*2*).

Accepting that there is the potential of exposure to a large number of neurobehavioral toxins, how does one begin to test for neurobehavioral toxicity? There is a perception in the toxicology community that the field of neurobehavioral toxicity testing is characterized by major methodological disagreements to the extent that it is impossible to initiate testing for regulatory purposes at this time. However, if one reviews the recommendations of various panels that have been assembled to develop protocols for neurobehavioral toxicity testing, as is shown in Table 3, it becomes clear immediately that certain tests have been consistently recommended for the past decade. Although some individuals or groups have recommended more extensive testing than others, Table 3 shows that there is almost unanimous agreement that screening for neurobehavioral toxicity should involve measurements of motor activity and schedule-controlled behavior, as well as neuropathology at the level of light microscopy with *in vivo* perfusion of the exposed subjects.

Motor activity is a measure of the animal's movement in a controlled environment. There are a large number of devices that have been developed to detect motor activity (*3*), including photocells, stabilimeters, field detectors, and running wheels, among others. At this time there is no strong consensus for any particular mea-

Table 3. Tests recommended for neurobehavioral toxicity testing.

Selected committee	Recommended tests
NAS/NRC (*19*)	Circadian cycle of motor activity Schedule-controlled behavior
NAS/NRC (*1*)	Unconditioned behavior (motor activity) Conditioned behavior (schedule-controlled behavior) Neuropathology with *in vivo* perfusion
Public Health Service Task Force (*20*)	Sensory function Motor function Learning and memory Performance (schedule-controlled behavior) Social behavior Affective behavior
EPA (*21*)	Schedule-controlled behavior Functional observational battery Neuropathology Peripheral nerve function Motor activity Neurotoxic esterase assay Acute-delayed neurotoxicity (organophosphates) Subchronic delayed neurotoxicity (organophosphates)
Wood (*18*)	Tier 1 Neuropathology Motor activity Schedule-controlled behavior Functional observation battery

surement system, as all of them appear to generate reproducible results under standardized conditions and the behavior measured is sensitive to disruption by toxicants. The automated measurement of patterns of motor activity in rodents has been particularly useful in behavioral toxicology, as it allows the continuous noninvasive measurement of the effects of a chemical on a stable behavior over long time periods (*4,5*).

Schedule-controlled behavior is behavior controlled by its consequences. In the usual situation in behavioral toxicology, a food-deprived animal is trained to press a lever to obtain food. Lever press responses produce food intermittently according to a schedule of reinforcement, which specifies the relationship between responses and the food availability. After a period of training, which may require a few days to a few months depending on the complexity of the reinforcement schedule, the behavior baseline shows low day-to-day variability against which to measure the effects of chemicals. This model has been employed widely to study the effects of drugs (*6*), as well as environmental toxicants. Like motor behavior, schedule-controlled responding permits the noninvasive measurement of the effects of a chemical on behavior over a long time period. It has the advantage over motor behavior in that behavior can be controlled and specified by the experimenter. With ingenuity, schedule-controlled behavioral procedures can be developed to measure specific functions such as learning, memory, sensory thresholds, etc. The major disadvantage of the procedure is that the training time under most schedules is prolonged relative to that for motor behavior. Although few systematic studies are available, the literature suggests that motor behavior and schedule-controlled behavior have the same order of magnitude of sensitivity to toxic insult (*7*), although a given test may be more sensitive to the effects of one chemical than another.

Perhaps the most serious form of neurobehavioral toxicity is produced by those chemicals that produce morphological damage to the CNS. Measurement of morphological changes in the CNS requires *in situ* perfusion of the animal and the use of contemporary methods of preparation of the tissue for examination by light microscopy and preferably by electron microscopy as well. Appropriate procedures have been discussed by Spencer and Schaumburg (*8*) for assessing the location, type, and degree of neurotoxicity. For the neurotoxins that have been investigated thus far in animal models, there is a high correlation between the type of damage produced in the models and the type of damage produced in humans following accidental exposure.

To illustrate how these recommended neurobehavioral toxicity tests might be used in regulatory risk assessment, an example will be developed. Before reviewing the data base to be used in risk assessment, it is necessary to review the risk assessment model to be used. Although a number of sophisticated models are under development, perhaps the one most widely employed at present is that of the United States Environmental Protection Agency (EPA) for issuing health advisories (*9*). The EPA has used the idea of an acceptable daily intake (ADI) over a lifetime using safety factors as first suggested by Lehman and Fitzhugh (*10*) and later modified by NAS (*11,12*).

Under this model, a no-observed-effect level (NOEL), a no-observed-adverse effect level (NOAEL), or a lowest observed adverse effect level (LOAEL), is determined on the basis of data collected during a complete review of the literature. The NOEL, NOAEL, or LOAEL is then divided by a safety factor of an uncertainty factor to allow for extrapolation to the human population as shown in Table 4. The uncertainty factors are applied in a series of steps based on a somewhat arbitrary factor of 10. For example, the NOEL or NOAEL is divided by 10 to account for intersubject variability within the human population when adequate human data are available. A second 10-fold uncertainty factor is applied for extrapolation across species when human data are not available. A third uncertainty factor of 10 is applied for extrapolation when less than chronic exposure data on animals are available in the absence of useful human data. A final uncertainty factor of 1 to 10 is used when the data available are from a lowest-observed adverse effect level, rather than a NOAEL, although in practice this factor is almost always 10. Thus, in a case where only subchronic animal data are available for the identification of a LOAEL, the mg/kg exposure representing the LOAEL is divided by 10,000 ($10 \times 10 \times 10 \times 10$) to develop the ADI. Whether or not an uncertainty factor of 10 is really appropriate at any of these stages of extrapolation has not been well documented. Although data allowing comparisons across species may be derived from the literature for some chemicals, determinations as to the variability of response across individual animals may be difficult to retrieve from the usual types of data reported.

The class of compounds to be subjected to risk assessment under this model are the alkyltins. Alkyltin compounds have been used as stabilizers in plastics, wood preservatives, disinfectants, pesticides, and for a variety of other industrial applications (*13*). Although many alkyltin compounds exist, the neurobehavioral

Table 4. Guidelines for the use of uncertainty factors.[a]

Guideline	Uncertainty factor
Extrapolation from valid data on prolonged ingestion by man to protect sensitive members of the population	10
Extrapolation from valid data on prolonged ingestion by animals in the absence of similar human data to extrapolate from the average animal to the average man	10
Extrapolation from valid data on acute or less prolonged ingestion by animals in the absence of prolonged ingestion by man or animals to extrapolate from less than chronic to chronic exposure	10
Extrapolation from a LOAEL to a NOAEL	1–10

[a] Data are from Ohanian and Fenner-Crisp (*9*).

toxicity of triethyltin (TET) and trimethyltin (TMT) have been studied most widely. TET produces fluid accumulation in myelin layers, resulting in a splitting of the myelin and severe cerebral edema, while TMT produces neuronal death, particularly in the hippocampus, but elsewhere as well (*13*).

Table 5 shows some representative studies indicating effects of TMT on motor activity. It is obvious from the table that mice and pigeons are more sensitive than rats to the effects of TMT on motor activity. Table 6 shows representative studies for the effects of TMT on schedule-controlled responding. Pigeons and rhesus monkeys are more sensitive to the effects of TMT than are rats and mice. Table 7 shows representative studies for neuropathological effects of TMT. Again, mice and pigeons appear to be more sensitive to the effects of TMT than rats.

On the basis of the data in Tables 5, 6, and 7, a LOAEL of 0.5 mg/kg can be established for the monkey (*14*) and a NOAEL of 0.3 mg/kg for the pigeon (*15*). Although there is a temptation to rely on primate data, preference for a NOEL over a LOAEL suggests the use of the pigeon data in calculation of the ADI and leads to the following calculation:

$$\frac{0.3 \text{ mg/km}}{10 \times 10 \times 10} = 0.0003 \text{ mg/kg} \quad [1]$$

If one assumes a 70 mg/kg adult human, the ADI, or reference dose, for man is:

$$70 \times 0.0003 = 0.021 \text{ mg/day} \quad [2]$$

If one uses the monkey data, an additional safety factor of 10 is required to convert the LOAEL to a NOEL which results in a lower ADI as shown below:

$$\frac{0.5 \text{ mg/kg}}{10 \times 10 \times 10 \times 10} \times 70 \text{ kg} = 0.0035 \text{ mg/kg} \quad [3]$$

In a similar manner, on the basis of data from McMillan and Wenger (*13*), one can calculate an ADI for TET. In contrast to TMT, where rats were relatively insensitive, rats are more sensitive to TET than mice. Pigeons are about as sensitive to TET as rats. A NOAEL of 0.3 mg/kg can be derived for the pigeon and a LOAEL of 0.5 mg/kg for the rat, which would result in the calculation of ADIs for TET identical to those for TMT (e.g., 0.0035–0.021 mg/day). If one adopts the most conservative point of view, an ADI for both of these alkyltins would be 0.0035 mg/kg/day.

Although there have been a few human exposures to alkyltins, it is difficult to determine the dose to which they were exposed. The only reference point in the literature comes from the ACGIH air standard, where "organic compounds as tin" (*16*) have a TLV of 0.1 mg/m^3 (based on 8 hr exposure 5 days per week). In order to compare the ADI determined from neurobehavioral toxicity testing with the ACGIH total limit value, it is necessary to convert this air quality standard to a milligram per kilogram dose absorbed. To make this conversion involves a number of assumptions of dubious validity, although it is a type of conversion sometimes made by regulatory agencies when it is necessary to develop a water quality standard with only air quality data available. Assuming 7 days a week exposure, Equation 4 can be used to make the calculation.

$$\frac{\text{mg/m}^3 \times 8 \text{ hr in m}^3 \times \text{week exposure} \times \text{absorbed}}{\text{Body weight}} = \text{mg/kg/day} \quad [4]$$

Unfortunately, data on pulmonary absorption percentages are not available for alkyltins. For purposes of making the calculation, an untested assumption of

Table 5. Effects of trimethyltin on motor activity in various species.

Animal	LOAEL or NOEL, mg/kg[a]	Effect[b]	Reference
Rat	6(N)	None	(*22*)
	5(L)	↑ then ↓	(*23*)
	3(L)	↓	(*24*)
	3(N)	None	(*25*)
Mouse	1(L)	↓	(*26*)
Pigeon	1(L)	↓	(*27*)

[a] (L), LOAEL; (N), NOEL.
[b] Increased activity (↑); decreased activity (↓).

Table 6. Effects of trimethyltin on schedule-controlled behavior in various species.

Animal	LOAEL or NOEL, mg/kg[a]	Effect[b]	Reference
Rat	6(L)	↑ Maze errors	(*28*)
	3(N)	↓ FR rate	(*24*)
	3(N)	↓ FI FR rate	(*29*)
	6.6(L)	DRL disrupted	(*30*)
Mouse	1(L)	↓ FI FR rate	(*31*)
Pigeon	0.3(N)	↓ FI FR rate	(*15*)
Rhesus monkey	0.5(L)	↑ Matching errors	(*14*)

[a] (L), LOAEL; (N), NOEL.
[b] Increased activity (↑); decreased activity (↓); FR, fixed ratio; FI, fixed interval; DRL, differential reinforcement of low rates.

Table 7. Neuropathology of trimethyltin in various species.

Animal	LOAEL or NOEL, mg/kg[a]	Area of effect	Reference
Rat	8(L)	Hippocampus	(*32*)
	7.5(L)	Hippocampus	(*33*)
	5(L)	Hippocampus	(*34*)
	7(L)	Brainstem, spinal cord	(*35,36*)
	6(L) (chronic neonate)	Peripheral nerve	(*37*)
	1(L)		(*38*)
Mouse	1(N)	None	(*23,28*)
	3(L)	Hippocampus, spinal cord	(*39,40*)
Pigeon	0.3(N)	None	(*15*)
Marmoset	3(L)	Hippocampus	(*41*)

[a] (L), LOAEL; (N), NOEL.

30% absorption of an inhaled dose was made. The calculation is as follows:

$$\frac{0.1\ m^3 \times 13.8\ m^3 \times 5/7 \times 0.3}{70} = 0.0042\ mg/kg/day \quad [5]$$

It can be seen that the ADI calculated from the conversion of the ACGIH total limit value is of the same order of magnitude as that developed from the animal model (e.g., 0.0042 versus 0.0035). Obviously these are crude estimates with many problems in addition to the conversion of inhalation data to parenteral administration effects to calculate an ADI. For example, the ACGIH total limit value does not mention the specific alkyltin, and it is well known that there is wide variation in toxicity among alkyltins (*17*). Furthermore, the animal data are derived from doses calculated as the salts, whereas the total limit value appears to refer only to the tin molecule, although this point is not absolutely clear. Nevertheless, it is interesting to note how closely the neurobehavioral ADI matches that derived from the ACGIH data.

At this time, comprehensive reviews of neurobehavioral toxicity data bases have not been done, so it is unclear as to what extent neurobehavioral ADIs relate to current regulatory standards, or perhaps the thesis should be stated another way to say that it is unclear to what extent neurobehavioral test results might modify current standards. At any rate, there is a consensus support that at least some neurobehavioral test methods are ready to be added to current test batteries for regulatory purposes.

The remainder of this paper will be devoted to a short discussion of some of the important questions facing the field of neurobehavioral toxicity today. Some of these questions are common in other areas of toxicity testing, while others are especially applicable to neurobehavioral toxicity testing.

Special Issues in Neurobehavioral Toxicity Testing

How Does One Define an Adverse Behavioral Effect?

Cancer is an adverse effect by definition. There are few who would argue that a malignant tumor is beneficial. With chemically induced behavioral changes, the case is less clear. For example, is an increase in motor activity an adverse effect? Does an increase in the rate of lever pressing, which in some cases may result in a hungry animal earning more food in less time, represent an adverse effect? Does increased accuracy in a delayed matching-to-sample task (frequently considered to be a measure of short-term memory) represent an adverse effect? Until a clear basis develops for considering some chemically induced behavioral changes as adverse and others not adverse, it would seem prudent to consider all behavioral changes caused by chemicals as adverse effects. If this viewpoint is adopted, the common progression from NOEL to NOAEL to LOAEL is inappropriate because the concept of a NOAEL is not employed. For this reason only NOELs and LOAELs were used in Tables 5, 6, and 7. This argument is easily defended when a chemical produces neuropathology, since most neuropathologists would agree that lesions are by definition adverse effects, but the issue can be reopened if the lesions do not produce measurable functional consequence.

Why Do Chemicals Produce Neurobehavioral Toxicity That Differs Across Species?

Differences in neurobehavioral responses to chemicals in different species appears in most instances to be quantitative rather than qualitative. It seems likely that most, if not all, such species differences will be related to pharmacokinetic factors rather than to fundamental differences in structure and function of the nervous system. Unfortunately, neither the pharmacokinetic nor the neurobehavioral data are sufficiently available to make a reasonable test of this assumption. Correlations between dose-time-response data for neurobehavioral changes with the uptake, distribution, metabolism, and excretion of toxic chemicals are badly needed.

Must Chemicals Act Directly on the CNS to Produce Behavioral Toxicity?

It is obvious that some chemicals can produce profound behavioral changes without ever reaching the brain. For example, some chemicals stimulate nerve endings of fibers that transmit pain sensation to the brain. Although such chemicals are active in behavioral tests, they are not neurobehavioral toxins in the usual sense. In order for a chemical to produce peripheral effects of sufficient magnitude to activate the CNS indirectly and result in behavioral changes, considerable peripheral activity must occur. Whether such peripheral effects are more easily and reliably directly measured than are the behavioral changes they may produce is an unanswered question. It may be that behavioral changes can function as a noninvasive measure of the general well being of the organism.

Is There a Correlation Between CNS Damage and Behavioral Change?

Considerable energy has been expended by neuropathologists and behavioral toxicologists arguing about whether one method is more or less sensitive than the other. Although the answer to the question may have some regulatory significance, it probably has little scientific importance. One point should be remembered. A large number of chemicals that produce profound behavioral toxicity produce no apparent lasting morphological damage. Carbon monoxide, many psychoactive drugs, perhaps even lead, fit into this category. Until

a larger data base is developed, it seems appropriate to recommend both behavioral testing and neuropathology testing when we know little about the toxicity of a chemical.

Alternative Methods and Animal Rights

Certain types of toxicity testing such as the LD_{50} have come under considerable criticism, especially from animal rights groups, in part because such large numbers of animals have been used. A major advantage of carefully controlled behavioral experiments is that they reduce the number of animals required for toxicity testing to a minimum. Rigid experimental control is substituted for the statistical control necessary with large groups. What may be lost in this approach is the ability to detect effects that occur infrequently in large populations, if in fact neurobehavioral toxicity occurs in this manner.

At this point in time it seems impossible to eliminate the need for the use of whole animals in neurobehavioral toxicity testing. Although tissue culture techniques have shown some utility in neurotoxicity testing, the likelihood that a simple system of this type could predict all of the subtlety of behavior is unlikely. The whole organism modifies its behavior as a function of its experience (learns) and acts appropriately when faced with situations to which it has been previously exposed (remembers). That these and other complex behavioral processes could be modeled in tissue culture or in computer program in the near future is difficult to imagine. This does not mean that such research attempts should not proceed, but even when promising data from these attempts accumulate, they will have to be validated in the whole organism.

Another alternative method is measurement at the physiological level. For example, some chemicals release neurotransmitters, activate or block receptors, or directly change nerve cell permeability while the chemical is present at the target site. Yet, as these chemicals are metabolized and/or excreted, these functional effects disappear. Such functional changes may produce behavioral changes, or at least be precursors of behavioral change; yet, until recently, such effects could not be measured noninvasively. With some of the new imaging techniques available, such as nuclear magnetic resonance, the situation may be changing and functional changes in the activity of the nervous system are likely to be accessible in the near future. This is clearly a growth area in neurobehavioral toxicity for the next decade.

Quality of Life

Wood (*18*) has recently raised the issue of quality of life as a neurobehavioral toxicity concern. For example, certain chemicals may not damage the CNS or produce obvious behavioral deficits, yet they cause us to change our lives to avoid exposure to the chemical. Perhaps a good example of this nebulous concept is to consider the matter of odor. People do not remain in environments where there are bad odors unless other factors leave them little choice. If someone sells a home at a financial loss because of its proximity to a paper mill that emits an offensive odor, the chemical producing the odor has caused an important behavioral change, even though it is not neurobehaviorally toxic in the usual sense of the word. Quality of life is a particularly difficult issue for risk assessment, especially when only animal data are available, yet it is an area where neurobehavioral toxicologists need to begin to direct their attention.

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